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EXPERIMENTAL STUDY ON THE DRAINAGE OF SO-CALLED INTRAVENOUS FAT PIGMENT FROM THE LIVER. LONG-TERM OBSERVATION WITH HISTOLOGIC, ENZYME-HISTOCHEMICAL AND ELECTRON MICROSCOPIC ANALYSIS

BY

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ABSTRACT

The drainage and decrease of so-called intravenous fat pigment (i.v. fat pigment), which was a lipoid-pigment complex and deposited in the reticuloendothelial system after repeated infusions of Intrafat (intravenous fat emulsion), was observed in the liver of the rabbit by histological, histochemical and electron microscopic methods.

As a result, the i.v. fat pigment was mainly found in Kupffer cells immediately after the repeated injections of Intrafat. After then, depending on the period when no injection were given, it decreased gradually and disappeared finally from Kupffer cells. On the other hand, in the interstitium of Glisson's sheath, many phagocytes, extremely swollen and laden with i.v. fat pigment, appeared during the postinfusion period. Furthermore, these phagocytes laden with i.v. fat pigment were found in Glisson's sheath for a long period, although they showed a tendency of decrease. Histochemical and electron microscopic characteristics, quite similar to those of Kupffer cells, suggested that these phagocytes corresponded to the extremely swollen histiocytes or macrophages having some role in the drainage or the metabolism of the i.v. fat pigment.

INTRODUCTION

The so-called intravenous fat pigment (i.v. fat pigment), used in this study, is a lipoid-pigment complex, possibly a peroxidated lipoid substance [1, 2], which is different definitely from the other kinds of pigment in the living body and which deposits in Kupffer cells of the liver after repeated intravenous injections of Intrafat [1, 3] (a fat emulsion, DI-22, fat emulsion for intravenous injection developed by the Daidoh Eiyo Co., Ltd., Tokyo, consisting of soya bean oil 10.0 g, phospholipid 1.2 g, glycerol 2.5 g aq. dest. ad. 100 ml). It is still unknown whether this kind of lipoid-pigment complex has any influence on the living body, particularly on the reticuloendothelial system, and what kind of metabolism or drainage it receives in the organs.

When we collected the human autopsy cases [2], who had received repeated infusions of Intrafat to obtain high calories, and histopathologically observed them, we noticed a tendency that the i.v. fat pigment decreased gradually from Kupffer cells in the liver in relation to the period when there was no Intrafat infusion. Furthermore, there were many i.v. fat pigment laden-cells in the interstitial space of Glisson's sheath (Fig. 1). In order to learn how

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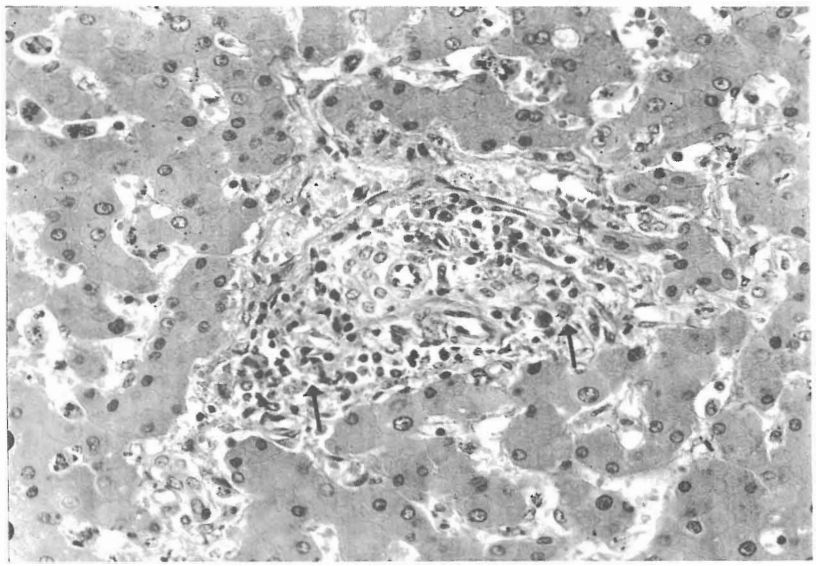

Fig. 1. Light microscopic photograph of the human liver in an autopsy case (52 y/o, male), who received infusions of Intrafat (500 ml/day×total 89 days). Postinfusion period to the death was one day. This case was carcinoma of the colon with no metastasis to the liver. Laboratory data at the terminal stage: GOT 49, GPT 23 (K. A. Unit), Al-P 23, total cholesterol 190, LDH 340. Treatment: Subtotal colectomy. 5-FU, total 18.75 g. Deposition of so-called i.v. fat pigment is seen in the swollen Kupffer cells, and the pigment-laden phagocytes (arrows) are occasionally found in the interstitial space of Glisson's sheath. Hematoxylin-eosin staining, ×300.

the i.v. fat pigment decreased from the liver and how these i.v. fat pigment-laden cells appeared in the interstitial space of Glisson's sheath, an experimental study on the Intrafat-infused rabbits was conducted particularly to study the decrease and the change of localization of this i.v. fat pigment and i.v. fat pigment-laden cells in the liver. As a result, a clear relationship between the decrease of the i.v. fat pigment in the liver and the period when there was no infusion of Intrafat was found and summarized in the present paper. The characteristics and role of the i.v. fat pigment-laden cells in Glisson's sheath were also analysed as a sort of drainage or metabolizing system of the pigment.

Materials and Methods

Adult female rabbits (10–11 weeks old) were infused with Intrafat by the following program. Group 1: Repeated injections of Intrafat (1.5 g/body weight kg/day) every day for three months were given and the rabbits were sacrificed three days after the last injection. Groups 2, 3, 4, and 5: Repeated injections of Intrafat every day for three months were given and the rabbits were sacrificed 6, 9, 12, and 19 months after the last injection, respectively. Groups 6 and 7: Untreated rabbits of the same age were used for controls. Each group consisted of two to five rabbits, except for groups 5 and 7 which consisted of only one rabbit, in each group.

Routine histological examinations were performed on each organ. Oil red 0 and Sudan III stainings were done on the cryostat liver sections after fixation with buffered formalin. Several kinds of histochemical examinations were done on the cryostat liver sections as follows: α-

naphthyl-acetate esterase and acid phosphatase by Leder [4], L(+) tartrate-resistant acid phosphatase by Yam [5] and alkaline phosphatase by Stutte [6].

Electron microscopic examination was also performed as follows: The liver was fixed with 2.5% glutaldehyde in a phosphate buffer solution (0.1 M, pH 7.2) and later fixed with 2% OsO_4 in a phosphate buffer solution. After epon and araldite embedding, the ultrathin sections were stained with uranium acetate and lead citrate. After glutaldehyde fixation acid phosphatase was also made to react on the thin sections before embedding and only the uranium acetate staining was done on the ultrathin sections after epon and araldite embedding. Each specimen was observed by the Akashi LEM-2000 electron microscope.

Results

In this experimental study using rabbits, the relationship between the post-infusion period of Intrafat and the deposition of the i.v. fat pigment in the reticuloendothelial system, particularly in the liver, was quite similar to the clinicopathological data of the human autopsy cases already reported [2], as shown in Table 1. In group 1 where repeated injections of Intrafat were given for three months and the rabbits were sacrificed three days after the last injection, deposition of the i.v. fat pigment in Kupffer cells was the severest among the experimental groups (Fig. 2). But, the i.v. fat pigment-laden cells in Glisson's sheath were relatively scarce. As shown in groups 2, 3, 4, and 5, the i.v. fat pigment decreased from Kupffer cells, depending on the period when there was no infusion of Intrafat, and finally disappeared. On the other hand, in Glisson's sheath, the i.v. fat pigment-laden and the cells swollen with lipoid substance increased and became prominent, particularly in group 4 as shown in Fig. 3. According to the lipid staining and electron microscopic observation, the cytoplasms of these pigment-laden cells in Glisson's sheath were extremely swollen with lipoid substance including the i.v. fat pigment (Figs. 4, 5, and 6).

Histochemical analysis showed that the pigment-laden cells in Glisson's sheath had quite similar characteristics to those of Kupffer cells as described in Table 2. Cells of both types had a large amount of lipid, clearly positive by Oil red 0 (Fig. 4) and Sudan III stainings in the cytoplasm. The strong positive reaction of acid phosphatase in addition to α-naphthyl-acetate

Table 1. Relationship Between Dosage and Period When There was no Infusion of Intrafat and the Change of Intrahepatic Deposition of i.v. Fat Pigment in Rabbits

Group	Duration of[a] infusion of Intrafat	Number of months between termination of Infusion and death	Quantity of i.v. fat[b] pigment in Kupffer cells	Pigment and lipid[c]-laden phagocytes in Glisson's sheath
1	3 months	0 (3 days)	+++	+
2	3	6	+	+
3	3	9	+	++
4	3	12	+	++
5	3	19	−	++
6	0	12	−	−
7	0	19	−	−

a Repeated injections of Intrafat (1.5 g/body weight kg/day) every day were given to adult female rabbits (10–11 weeks old).

b +++ Pigment can be seen often, ++ Occasionally seen, + Rarely found.

c +++ Phagocytes can be seen easily and often, ++ Occasionally seen, + Rarely found.

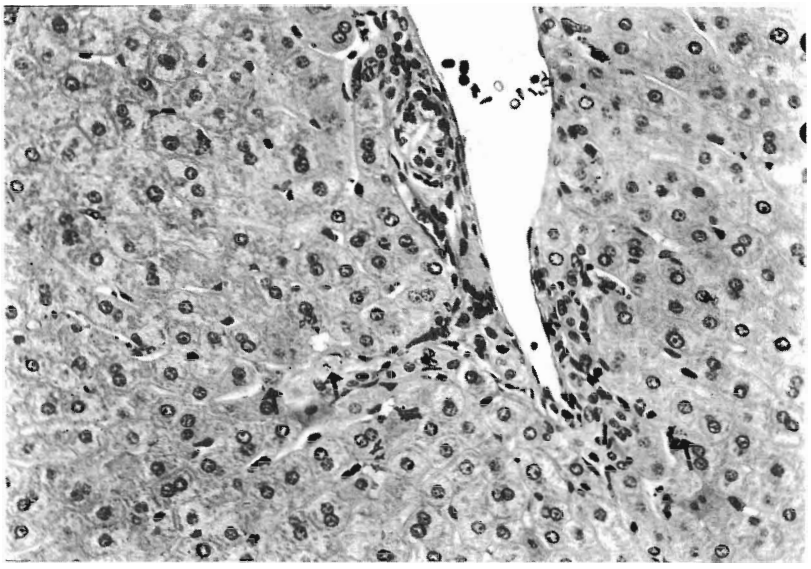

Fig. 2. Light microscopic photograph of the rabbit liver in experimental group 1. I.v. fat pigment is clearly deposited in the swollen Kupffer cells (arrows). On the other hand, pigment-laden cells are rarely found in Glisson's sheath. Hematoxylin-eosin staining, ×300.

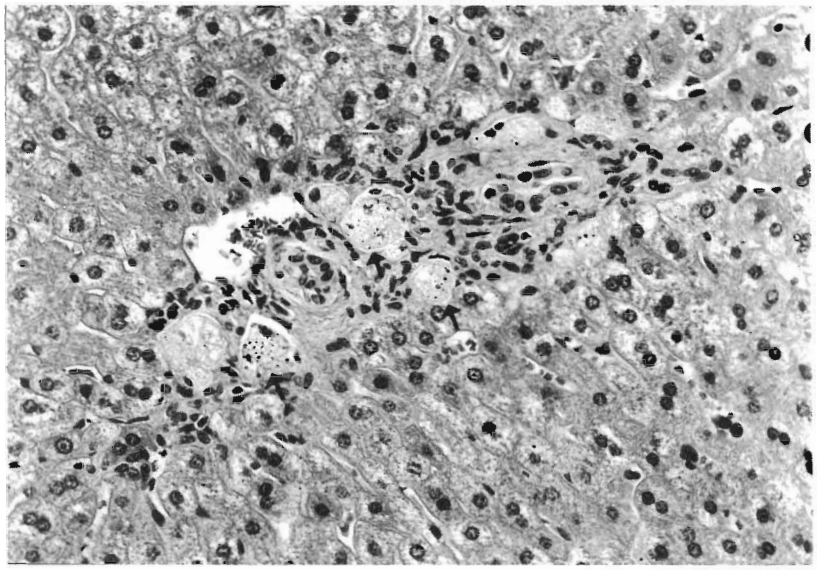

Fig. 3. Light microscopic photograph of the rabbit liver in experimental group 3. In comparison with the decrease of i.v. fat pigment-laden Kupffer cells, many lipid and pigment-laden phagocytes accumulate in or around Glisson's sheath (arrows). Hematoxylin-eosin staining, ×300.

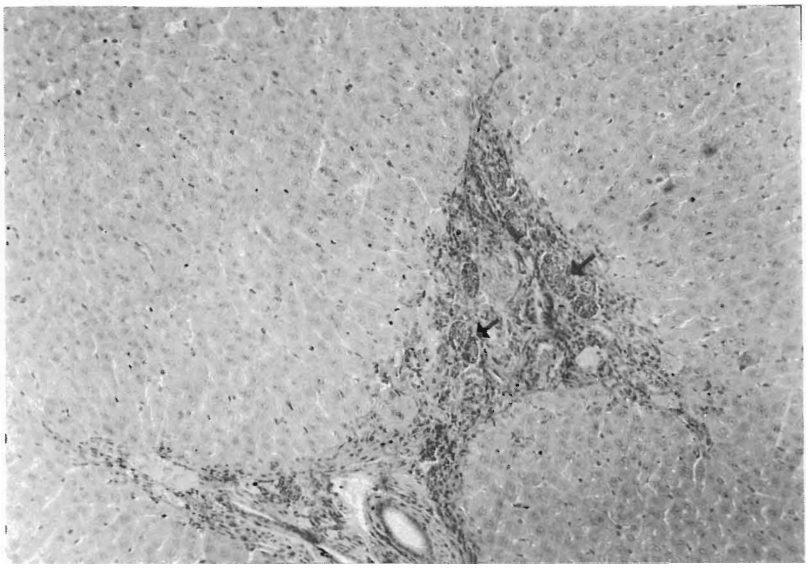

Fig. 4. Light microscopic photograph of the rabbit liver in experimental group 4. The swollen cytoplasm of the i.v. fat pigment-laden phagocytes in Glisson's sheath show a strong positive reaction by lipid staining (arrows). Oil red 0 staining, ×120.

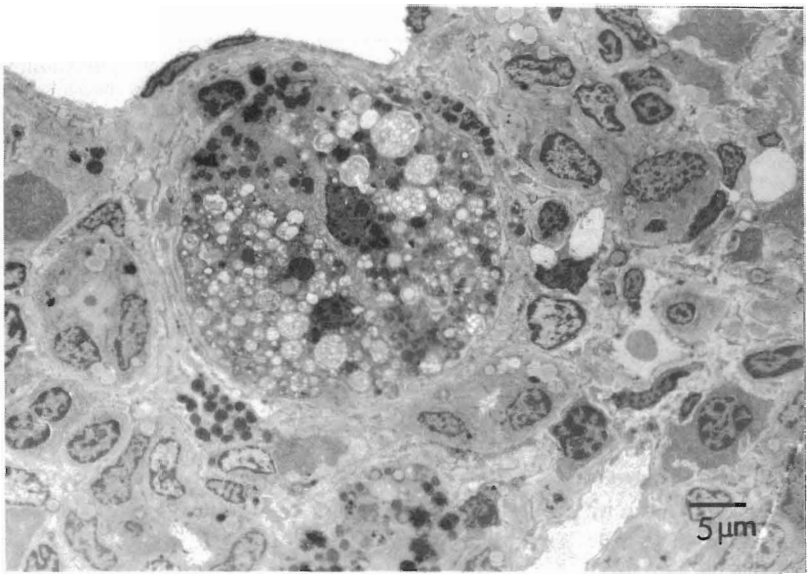

Fig. 5. Electron microscopic photograph of the i.v. fat pigment-laden phagocytes in the interstitium of Glisson's sheath of the rabbit liver (experimental group 4). Two phagocytes, laden and extremely swollen with lipid and i.v. fat pigment, accumulate in the limited space. Double stainings by uranium acetate and lead citrate, ×1,500.

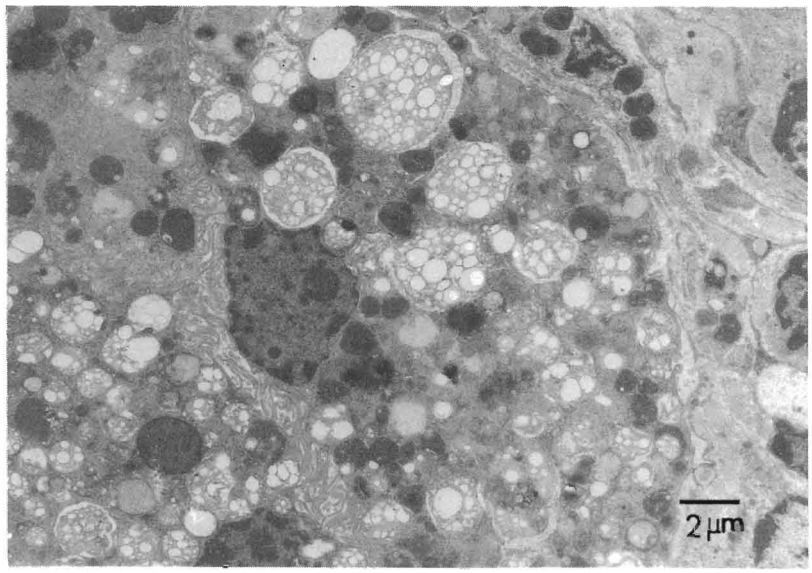

Fig. 6. Enlarged photograph of the same region as that of Fig. 5. Lipid and i.v. fat pigment of various sizes are clearly seen in the swollen cytoplasm of the phagocyte. Double stainings by uranium acetate and lead citrate, ×3,900.

Table 2. Enzyme-Histochemical Findings of Cells in Liver (Experimental Group 4)

Staining	Liver cell	Kupffer cell	Phagocytes in the interstitium of Glisson's sheath
Oil red 0	−	+	++
Sudan III	−	+	++
Acid phosphatase	−	++	++
L(+)-tartrate-resistant acid phosphatase	−	−	−
α-naphthyl-acetate esterase	++	+	+
Alkaline phosphatase	++	−	−

++ Strongly positive, + Positive, − Negative.

esterase revealed that these swollen cells corresponded to the phagocytes, similar to Kupffer cells. After the acid phosphatase reactions, lead depostion was seen on the limiting membranes of the lysosomes in both the intralobular Kupffer cells and the i.v. fat pigment-laden phagocytes within Glisson's sheath by electron microscopic observation (Fig. 7).

These phagocytes, swollen and laden with i.v. fat pigment, were found not only in Glisson's sheath but also in the lymph node, spleen and bone marrow. In group 5, the liver showed a tendency of decrease of phagocytes laden with i.v. fat pigment in Glisson's sheath as well as the disappearance of the i.v. fat pigment from Kupffer cells (Table 1). Accordingly, phagocytes laden with the i.v. fat pigment have also some role in the drainage and metabolism of the i.v. fat pigment via the interstitium of Glisson's sheath in the liver.

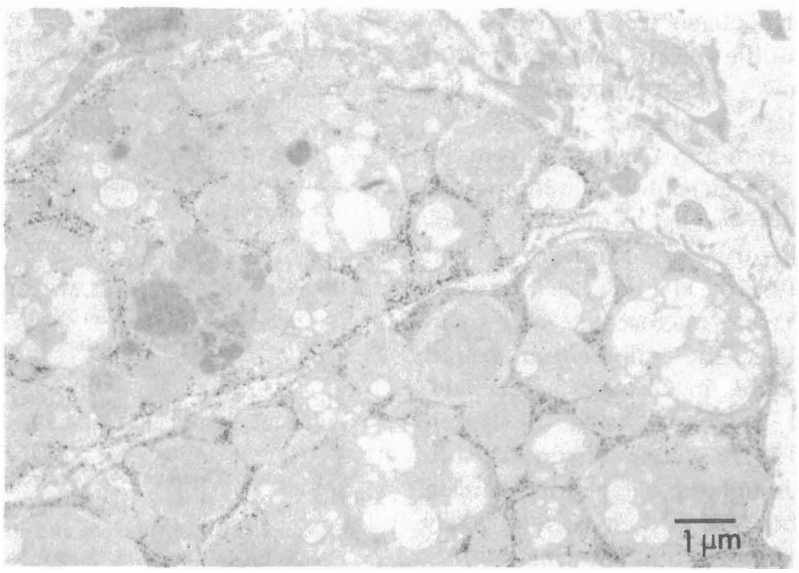

Fig. 7. Enzyme-electron microscopic photograph of the i.v. fat pigment-laden phagocyte in the interstitium of Glisson's sheath in the liver (experimental group 4). Many granules, as a result of positive reaction with acid phosphatase, are easily seen on the limiting membrane of the lysosomes in the swollen cytoplasm. Uranium acetate staining, ×7,900.

Discussion

Ever since intravenous fat emulsion was developed as a parenteral nutrition, it has been observed and discussed that the so-called i.v. fat pigment is deposited in the systemic reticuloendothelial system of the humans and animals by the infusion of large amounts of intravenous fat emulsion [1, 2]. Deposition of this pigment is observed in the organs without being metabolized for a long period [1]. It is not clear that this pigment, deposited in the reticuloendothelial system, receives any kind of drainage from the organs, particularly from the liver.

In this study, it was confirmed that this i.v. fat pigment decreased quite gradually in the liver, depending on the period when there was no administration of intravenous fat emulsion. It drained from Kupffer cells via the blood-vascular system based on the turnover of Kupffer's cells, probably due to shedding or migration [7, 8], as the major route. Further, as a minor route, the i.v. fat pigment could drain at the cellular level via the interstitium of Glisson's sheath, based on the turnover of the phagocytes, because of the following reasons. Several, ultrastructural and histochemical features of the i.v. fat pigment-laden phagocytes in Glisson's sheath were quite similar to those of Kupffer cells as follows: 1) Phagocytic storage of the i.v. fat pigment and lipid substance in the cytoplasm, as shown by the electron microscopic pictures. 2) Positive, lysosome-enzyme-histochemical reaction such as acid phosphatase in the cytoplasm. Accordingly, it is strongly suggestive that these i.v. fat pigment-laden phagocytes in Glisson's sheath had almost the same function against the i.v. fat pigment as Kupffer cells did. Because phagosomes were abundant in the cytoplasm, these pigment and lipid-laden cells could be definitely differentiated from the fat-storing cells (of Ito) [9].

The swollen phagocytes could be easily found also in the spleen, lymph node and bone marrow. Accordingly, the i.v. fat pigment-laden phagocytes in Glisson's sheath seemed to have originated from the histiocytes or macrophages, although it was quite controversial to decide and differentiate the origins of Kupffer cells and histiocytes [9, 10, 11, 12, 13]. Actually, minute electron microscopic oservation could not detect the endothelium around these phagocytes in Glisson's sheath, the presence of which suggested the lymph vessel [14, 15, 16] within Glisson's sheath. Anyway, although the detailed mechanism could not be described, it seemed likely that these phagocytes in the interstitium had some role in the metabolism and drainage of the i.v. fat pigment in the liver.

This work should be further pursued to learn what kind of a functional relationship exists between Kupffer cells in the intralobule and the phagocytes in Glisson's sheath of the liver and whether the origin of the cells of both types is the same or not at the next opportunity.

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